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# STUDIES ON THE SEDATIVE, HYPOTENSIVE AND BRADYCARDIC EFFECTS OF dl-TETRAHYDROPALMATINE IN THE RAT

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*Abstract*

The effects of dl-tetrahydropalmatine on sedation, cardiovascular function and striatal dopamine (DA) release were compared in rats. dl-THP significantly decreased the motor activity of rats and showed dose-response pattern. It produced rigidity at a higher dosage. dl-THP also enhanced the hypomotility induced by α-methyl-p-tyrosine, and haloperidol, but reduced the hypermotility produced by l-DOPA plus benserazide. Furthermore, dl-THP significantly decreased the concentration of norepinephrine (NE), dopamine (DA) in the cortex and brain stem at lower and higher doses, and the concentration of serotonin (5-HT) in the cortex at higher doses. dl-THP also increased the concentration of homovanillic acid (HVA) in the cortex and brain stem at lower and higher doses, and the concentration of 5-hydroxyindole acetic acid (5-HIAA) in the cortex at higher doses.

Intravenous administration of THP (1-10 mg/kg) produced hypotension, bradycardia, and increased DA release in the striatum and decreased hypothalamic serotonin (5-HT) release (measured by carbon-fiber electrodes in combination with voltammetry). In addition, the THP-induced hypotension was attenuated by pretreatment with spinal transection or amphetamine, while the THP-induced bradycardia was attenuated by pretreatment with bilateral vagotomy or amphetamine. Postsynaptic blockade of 5-HT2 receptors with cyproheptadine (2-5 mg/kg, i.v.) or ketanserin (2-5 mg/kg, i.v.) produced both hypotension and bradycardia, while stimulation of 5-HT2 receptors with 1-(2,5-dimethoxy4-iodophenyl)-2-aminopropane (DOI) (10-250 pg/kg, i.v.) produced both hypertension and tachycardia. The dl-THP-induced hypotension and bradycardia could be reversed by DOI treatment.

The fever induced by interleukin -1β (2 ng in 10 μl by lateral cerebral ventricle injection), LSP (200 ng in 10 μl by lateral cerebral ventricle injection) was attenuated by treatment or pretreatment with dl-THP (20 mg/kg, i.p.) in unanesthetized rats.

These results suggest that dl-THP could decrease central dopaminergic activity in the cortex and brain stem at lower and higher doses and serotonergic activity in the cortex at higher doses. The sedative effect of dl-THP at lower doses may be due to a decrease in

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central dopaminergic neurons. The rigid effect of dl-THP may be due to a decrease in the central monoaminergic system. dl-THP induces hypotension and bradycardia through DA $D_2$ receptor antagonism in the striatum and through a serotonergic release process in the hypothalamus in rats.

*Introduction*

*Corydalis yanhusuo* is used extensively by practitioners of Chinese herbal medicine both in Asia and the West, where it is recognized as a safe and often effective remedy for common pain syndromes, especially abdominal pain (Kin and Hsu, 1957; Zhu, 1987). dl-Tetrahydropalmatine (dl-THP), the main active constituent of the *Corydalis* tuber, belongs to the protoberberine type alkaloids and has been proven to possess analgesic, sedative, tranquilizing and slightly hypnotic effects (Dharmananda, 1994; Hsu and Kin, 1962; Hsu and Kin, 1964). The sedative mechanism of dl-THP is shown to be a brain monoamine depletor by HPLC-ECD (Liu, 1983). Liu investigated the sedative effect of dl-THP, only detecting the concentration of central monoamines and its metabolites (Liu, 1983). He suggested that dl-THP only at dosages greater than 60 mg/kg shows a sedative effect. However, he did not observe any behavioral changes in rats by behavioral pharmacological methods. Furthermore, based on our preliminary experiment, dl-THP at dosages less than 10 mg/kg has a sedative effect. This effect is the same as that of Liu. We also found that dl-THP had rigid effect in rats in higher dosages. However, because the sedative and rigid effects of dl-THP have never been previously studied by behavioral methods, the sedative and rigid effects of dl-THP and its action of mechanism are investigated in this study by measuring the motor activity and the concentrations of brain monoamines and its metabolites in rats.

Recently, central dopaminergic systems have been implicated in the regulation of cardiovascular function. For example, stimulation of dopaminergic neurons in the ventral tegmental area (Cornish and Van den Buuse, 1994) or the substania nigra pars compacts (SNC) (Lin and Yang, 1994) induces both hypertension and tachycardia in rats. Other lines of evidence also indicate that THP acts on the nigro-striatal neurons as a dopaminergic receptor antagonist (Marcenac *et al.*, 1986). These results indicate the possibility that THP acts through a central nervous dopaminergic mechanism to influence cardiovascular function. In the present study, in order to examine the underlying mechanisms of the dl-THP-induced cardiovascular response, experiments were carried out to assess the effects of dl-THP on both the cardiovascular function and striatal dopamine (DA) release in sham-operated rats, in spinal-transected rats, in vagotomized rats, and in amphetamine-treated rats. In addition, the cardiovascular responses by dl-THP were compared with those of haloperidol (a DA $D_2$ receptor antagonist) and apomorphine (a DA receptor agonist).

There is accumulating evidence indicating that serotonin (5-HT) has an important regulatory role in the central control of cardiovascular function (Gothert and Kolassa, 1990). The central serotonergic system acts through the sympathetic and parasympathetic

pathway activity to affect the vasculature and heart. Activation of a bulbospinal projection that descends from the ventrolateral medulla and terminates in the intermediolateral column evokes a pressor response (Minson *et al.*, 1990). Activation of one important ascending 5-HT pathway innervating the hypothalamus also causes an increase in blood pressure (Kuhn *et al.*, 1980). It has been shown that dl-THP depletes the levels of three neurotransmitters in the central nervous system: 5-HT, dopamine and norepinephrine (Liu *et al.*, 1982). This raises the possibility that dl-THP acts through a hypothalamic serotonergic mechanism to influence blood pressure. Therefore, the present study has attempted to assess the effects of dl-THP on both the hypothalamic 5-HT release measured by *in vivo* voltammetry, blood pressure and heart rate in rats. In addition, the cardiovascular responses by dl-THP were compared with those of DOI (a 5-$HT_2$-receptor agonist), ketanserin and cyprohetadine (two 5-$HT_2$-receptor antagonists).

## *Materials and Methods*

### *Animals*

Six male Sprague-Dawley rats weighing 200-300 rats were housed in a temperature-controlled room on a normal light (09:0021:00)/dark cycle and served as subjects. The rats had free access to food and water for at least 2 weeks before the experiments.

### *Drugs*

Drugs, administered intravenously, included dl-tetrahydropalmatine (dl-THP) (supplied by the institute of Applied Chemistry, Providence College of Arts and Sciences, Taichung, Taiwan), 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) (RBI, Natick, MA, USA), cyproheptadine (RBI) and ketanserin (RBI). dl THP was dissolved in a stock solution made of 10% (v/v) phosphoric acid plus distilled water (1: 9). The pH of the dl-THP solution was adjusted to 4.5 with 1 N NaOH.

α-Methyl-p-tyrosine (α-MT) was dissolved in 0.9% saline. Haloperidol (HAL, Sigma) was dissolved in 0.1 M lactic acid to a concentration of 1 mg/ml and further diluted with physiological saline. Apomorphine (APO, Sigma) was dissolved in ascorbic acid for injection.

### *Measurement of motor activity*

The motor activity of individual rats was measured using the MK-ANIMEX activity meter (Model SE. Muromachi Kikai Co., Japan). The sensitivity and tuning of the instrument was adjusted to 35 μA to enable all kinds of motor behavior, including locomotion, rearing, grooming, sniffing and licking to be jointly recorded. Activity was recorded for 2 hours, starting 5 mins after inserting the animal into the test cage. Each rat was used only once and a total of six rats were used for each treatment.

The following compounds were given before administration of dl-THP as following: α-MT (100 mg/kg, i.p. ) 2 hours before (Widerlov and Lewander, 1978a); HAL (0.01 mg/kg, i.p.) 30 mins before administration of dl-THP (Megens *et al.*, 1988), APO (0.5 mg/kg, s.c.) 20 mins before administration of dl-THP (Montanaro *et al.*, 1983).

*Quantification of monoamines and its metabolites by HPLC*

dl-THP was given intraperitoneally at a dosage of 10 mg/kg. Ten min later, the rats were decapitated and the whole brain was removed and separated into the cortex and brain stem. The concentrations of NE, DA, 5-HT and their metabolites (VMA, HVA, 5-HIAA) were measured by high performance liquid chromatography (HPLC) with an Electrochemical Detector (Shibuya *et al.*, 1982). HPLC Model M-45 (Waters Associates), Data Model M-730 (Waters Associates) and Electrochemical Detector Model LC-4B (Bioanalytical systems) were used.

*Voltammetric measurement - Preparation of the carbon fiber electrode*

A multiple carbon fiber (28 m in diameter; Avco, Lowell, MA, USA) was inserted into a pulled glass micropipette (20- 25 mm in length). The tip was cut, and then the carbon fiber was pushed out of the pipette tip. Electrical contact with the fiber was made using silver paste. The tip and blunt and of the pipette were sealed with cyanoacrylate adhesive (super glue). The entire surface of the pyrolytic carbon fiber was 12-mm-thick. To improve the sensitivity and selectivity of carbon fiber electrodes for catecholamines or serotonin, the electrodes were electrically pretreated as described previously (Lin and Yang, 1994; Gonon *et al.*, 1981). This treatment consisted of a direct current applied in two stages: 2.2 V for 30 sec in 0.1 mol/l H2SO4 and 2.2 V for 30 sec in 1 N HCl. The carbon fiber electrode was washed with distilled water. The tip of the carbon fiber electrode was immersed into a Nafion drop (10 l of a 5% solution; Aldrich Chemical, Milwaukee, WI, USA) four times. The Nafion-coated electrode was then dried at 60° C for 20 sec and used immediately for *in vitro* followed by *in vivo* experiments (Lin and Yang, 1994; Brazell *et al.*, 1987).

*Electrochemical analysis of the striatal release of DA*

Differential pulse amperometry was performed *in vitro* and *in vivo* with a Biopulse apparatus (Solea Tacussel, France) using the following scan parameters: imposed initial potential=-220 mV, imposed final potential= -70 mV, pulse cycle=25 sec, prepulse=70 msec, measuring pulse=40 msec and measuring potential = 40 mV. The sensitivity of the Nafion-coated carbon fiber electrode to catecholamines or serotonin in the concentration range of 200-800 nmol/l was determined using differential pulse amperometry in a temperature-controlled (37 ° C) water bath. Phosphate-buffered saline (0.1 M, pH 7.4) was used as the blank and solvent for the test solutions. To determine the sensitivity of the Nafion-coated electrodes (resistance=200, capacitance=0.0056 F) for DA over 3,4-dihydroxyphenylacetic acid (DOPAC), ascorbic acid, or uric acid, the ratio of the sensitivity of DA to DOPAC, DA to ascorbic acid, or DA to uric acid was calculated. Our electrodes were 345-1,377 times more sensitive to DA than to DOPAC, ascorbic acid, or uric acid. Our electrodes are insensitive to serotonin. In addition, the very low concentration of norepinephrine and epinephrine in the rat striatum makes the measurement of extracellular levels of the striatal DA easy in the present study. The

animals were anesthetized with urethane (1.4 g/kg, i.p.), held in a stereotaxic frame and implanted unilaterally with a Nafion-coated carbon fiber electrode in the anteior hypothalamus using the coordinates of Paxinos and Watson (1982). Auxiliary (silver wire) and reference (Ag/AgCl) electrodes were placed on the dura surface of the parietal skull. Differential pulse voltammograms were then recorded automatically every 2 sec.

*Electrochemical analysis of the hypothalamic release of 5-HT*

Differential pulse amperometry was performed *in vitro* and *in vivo* with a Biopulse apparatus (Solea Tacussel, France) using the following scan parameters: imposed initial potential=-100 mV, imposed final potential= +260 mV, pulse cycle=2 sec, prepulse=80 msec, measuring pulse=40 msec and measuring potential = 40 mV. The sensitivity of the Nafion-coated carbon fiber electrode to 5-HT in the concentration range of 200- 1000 nM was determined using differential pulse amperometry in a temperature-controlled (37 $^{o}$ C) water bath. Phosphate-buffered saline (0.1 M, pH 7.4) was used as the blank and solvent for the test solutions. To determine the sensitivity of the Nafion-coated electrodes (resistance=200, capacitance=0.0056 F) for 5-HT over 5-hydroxyindoleacetic acid (5-HIAA), the ratio of the sensitivity of 5-HT to 5-HIAA was calculated. Our electrodes were 200 times more sensitive to 5-HT than to 5-HIAA. Our electrodes are insensitive to dopamine. The animals were anesthetized with urethane (1.4 g/kg, i.p.), held in a stereotaxic frame and implanted unilaterally with a Nafion-coated carbon fiber electrode in the anteior hypothalamus using the coordinates of Paxinos and Watson (Paxinos and Watson, 1982). Auxiliary (silver wire) and reference (Ag/AgCl) electrodes were placed on the dura surface of the parietal skull. Differential pulse voltammograms were then recorded automatically every 2 sec. After concluding the experiments, 4 mA anodal d.c. was passed through the working electrodes for 10sec and the resultant lesions were located to identify the actual electrode site. All electrode placements were in the region of the hypothalamus as verified by standard histological procedures. Animals were perfused with physiological saline, followed by 10% formalin. The brains were removed, sliced on a freezing microtome and stained with formyl thionine.

*Surgey*

In preparing spinal trans-sections, the cervical vertebrae were exposed and complete transection was made with a spatula at the 7th cervical segment of the spinal cord (C7). Bilateral vagotomy was made at the neck region.

*Assessment of cardiovascular function*

A fine catheter was inserted into the femoral artery of rats. The catheter was connected via a Statham blood pressure transducer to a Gould four-channel polygraph for recording mean arterial and pulsatile blood pressure. The catheter was also used for blood sampling. Both the heart rate and blood gases were measured. Another polyethylene catheter was inserted into the femoral vein to inject drugs.

*Statistical analysis*

The numerical values cited in the text refer to the mean ± S.E.M. The mean arterial pressure was calculated as the diastolic pressure plus one-third of the pulse pressure (systolic-diastolic pressure). Student's t-test was used when only two groups were compared. ANOVA was used for factorial experiments and Dunn's procedure for *post hoc* multiple comparisons among means was used (Kirk, 1986). The criterion for statistical significance was set at the 0.05 level.

*Results*

The results are presented in the data section

*Effects of dl-THP on motor activity.*

As shown in Figure 1, dl-THP could produce a significant decrease in motor activity and show a dose-dependent response ($p < 0.05$ - $0.001$). However, it produced rigidity at a higher dosage (20 mg/kg, i.p.).

Rats treated with α-MT or HAL alone appeared to be behaviorally depressed. The hypomotility produced by α-MT or HAL was significantly augmented by dl-THP ($p <$ 0.0l) (Figure 2).

As shown in Figure 3, APO produced significant hypermotility. The hypermotility produced by APO was significantly reduced by dl-THP ($p< 0.01$).

*Effects of the concentrations of monoamines and its metabolites in the brain stem and cortex.*

As shown in Table I, dl-THP (10 mg/kg, i.p.) significantly decreased the levels of NE ($P < 0.0l$), DA ($p < 0.05$), and increased the levels of HVA ($p < 0.05$). Also, dl-THP (20 mg/kg, i.p.) significantly decrease the level of NE, DA and 5-HIAA ($p < 0.01$), and increase the level of HVA ($p < 0.01$) and 5-HIAA ($p < 0.01$) in the cortex.

As shown in Table II, dl-THP (10, 20 mg/kg, i.p.) significantly decreased the levels of NE ($p < 0.01$), DA ($p < 0.05$, 0.01), and increased the level of HVA ($p < 0.05$, 0.01) in the brain stem.

Table III summarizes the response of cardiovascular parameters, blood gases and striatal DA release produced by intravenous administration of vehicle or THP in rats under urethane anesthesia. Administration of dl-THP (1-10 mg/kg), but not vehicle, produced a dose-related fall in either mean arterial pressure or heart rate as well as a dose-related increase in striatal DA release. A typical example for this kind of response is shown in Figure 4. For a dose of 5 mg/kg of dl-THP, either the mean arterial pressure, heart rate or striatal DA release was altered immediately after dl-THP injection, and did not return to the pre-injection level even after 60 min. dl-THP treatment produced an insignificant change in either $PaCO_2$, $PaO_2$ or pH.

Table IV also shows the response of cardiovascular parameters produced by intravenous administration of dl-THP to rats with different preparations. It was found that spinal transection, but not bilateral vagotomy, almost completely abolished the dl-THP-

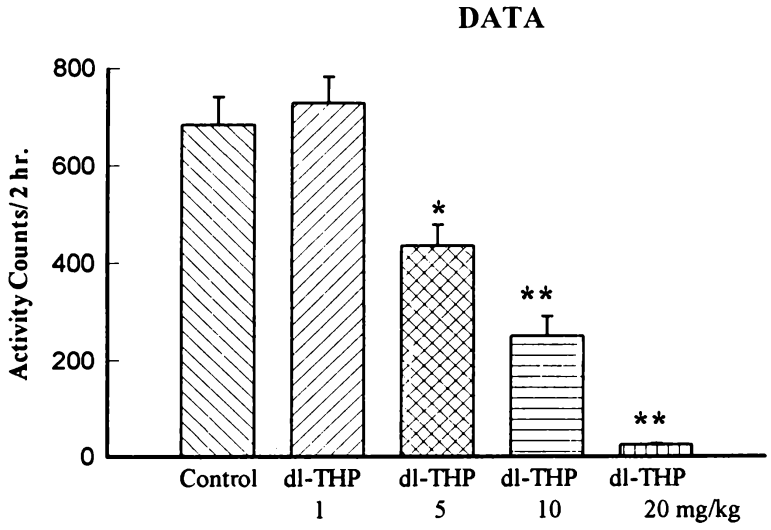


*Figure 1.* Effects of dl-tetrahydropalmatine on motor activity in rats. Value are mean ± SEM of six rats. Statistical comparisons of the dl-THP group vs. the control group use one-way ANOVA for repeated measures followed by Duncan's multiple range test (* $p < 0.05$, ** $p < 0.01$).

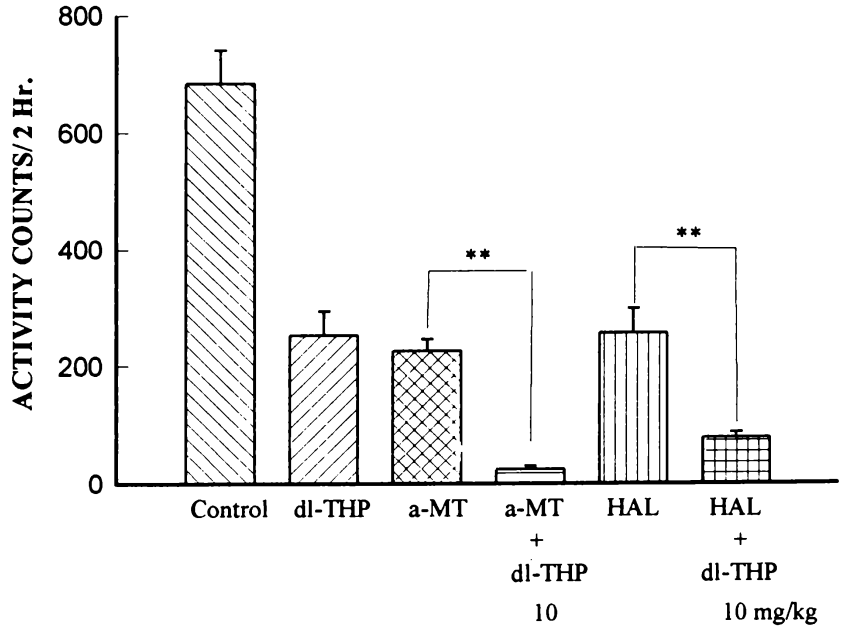


*Figure 2.* Effects of dl-tetrahydropalmatine on the motor activity produced by haloperidol in rats. Values are mean ± SEM of six rats. **$p < 0.01$ with respect to HAL group using one-way ANOVA for repeated measures followed by Duncan's multiple range test.

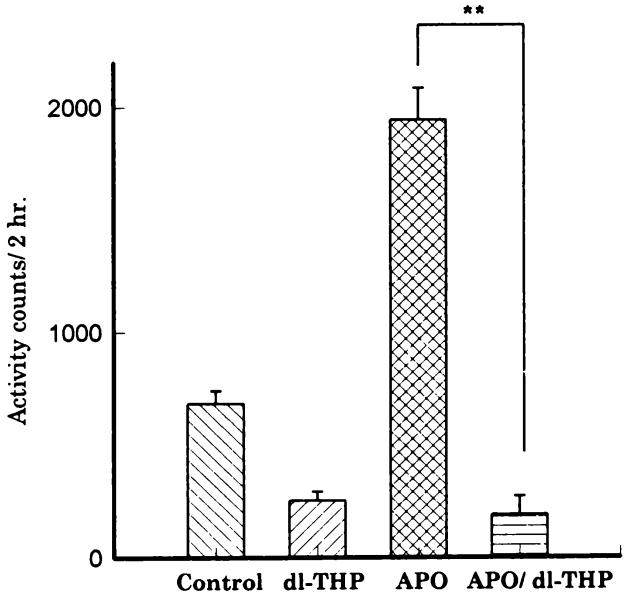


*Figure 3.* Effects of dl-tetrahydropalmatine on the motor activity produced apomorphine in rats. Values are mean ± SEM of six rats. **p < 0.01 with respect to APO group using one-way ANOVA for repeated measures followed by Duncan's multiple range test.

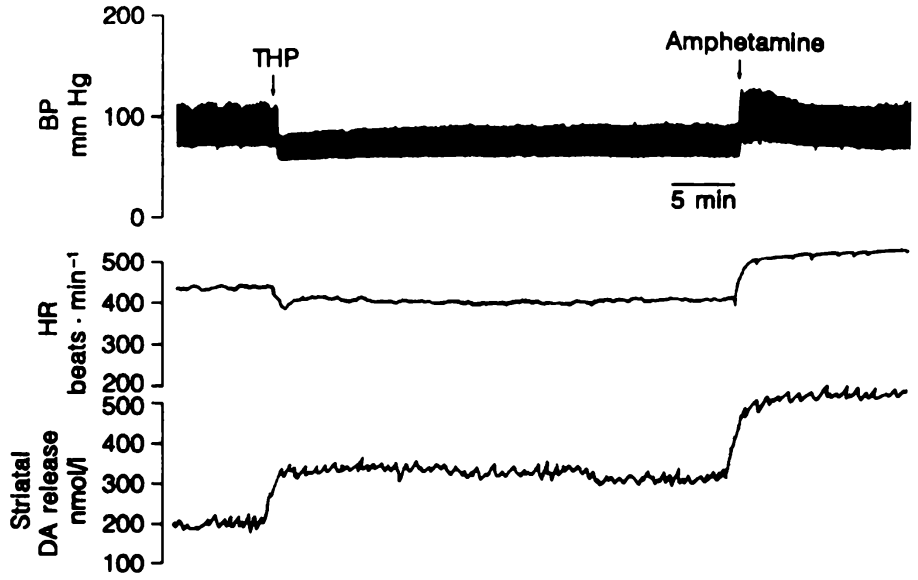


*Figure 4.* The time course changes in arterial blood pressure (BP), heart rate (HR), and striatal DA release produced by intravenous administration of dl-THP (5 mg/kg) or amphetamine (0.5 mg/kg) in an anesthetized rat.

**TABLE I**

The concentration (ng/g) of monoamines and its metabolites after acute treatment with dl-tetrahydropalmatine (THP; 10, 20 mg/kg; i.p.) in the cerebral cortex of rats.

| | NE | VMA | VMA/NE | DA | HVA | HVA/DA | 5-HT | 5-HIAA | 5-HIAA/5-HT |
|---|---|---|---|---|---|---|---|---|---|
| Control | 2567±305 | 325±19 | 0.127 | 2240±307 | 316±24 | 0.141 | 1079±72 | 424±17 | 0.393 |
| THP (10mg/kg) | 1663±91** | 341±27 | 0.205 | 1520±172* | 440±42* | 0.300 | 867±56 | 499±46 | 0.576 |
| THP (20mg/kg) | 981±80** | 380±24 | 0.388 | 368±78** | 501±34** | 1.363 | 663±81** | 596±42** | 0.900 |

Data are represented as the mean ± S.E. of 6 rats for each group in the above Table. * p value is compared with the control group by ANOVA following Duncan's multiple range test. * $p < 0.05$, ** $p < 0.01$.

**TABLE II**

The concentration (ng/g) of monoamines and its metabolites after acute treatment with dl-tetrahydropalmatine (THP; 10, 20 mg/kg; i.p.) in the brain stem of rats.

| | NE | VMA | VMA/NE | DA | HVA | HVA/DA | 5-HT | 5-HIAA | 5-HIAA/5-HT |
|---|---|---|---|---|---|---|---|---|---|
| Control | 1689±144 | 353±12 | 0.148 | 3666±373 | 370±26 | 0.101 | 794±60 | 315±47 | 0.398 |
| THP (10mg/kg) | 969±116** | 350±13 | 0.210 | 1031±209* | 483±34** | 0.469 | 681±32 | 338±27 | 0.496 |
| THP (20 mg/kg) | 615±98** | 374±13 | 0.284 | 700±45** | 557±36** | 0.796 | 654±52 | 380±45 | 0.581 |

Data are represented as the mean ± S.E. of 6 rats for each group in the above Table. * p value is compared with the control group by ANOVA following Duncan's multiple range test. * $p < 0.05$, ** $p < 0.01$.

**TABLE III**

Maximal changes in mean arterial pressure (MAP), heart rate (HR) and hypothalamic DA release produced by intravenous injection of dl-THP in anesthetized rats

| Treatment (mg/kg) | △MAP mmHg (n=8) | △HR beats/min (n=8) | △PaCO2 mm Hg (n=8) | △PaO2 mm Hg (n=8) | △Striatal DA release, nmol (n=8) |
|---|---|---|---|---|---|
| 0 | 4.8± 1.2 | -2.6± 0.5 | -2.5± 0.5 | -7.0± 2.1 | 4+1 |
| 1 | -13.8± 2.2* | -11.3± 2.3* | -3.1± 0.5 | -5.3± 1.4 | -21+4* |
| 5 | -20.6± 3.1* | -27.5± 5.3* | -3.4± 0.3 | -6.2± 1.3 | -39+5* |
| 10 | -29.4± 3.6* | -42.5± 6.5* | -3.6± 0.4 | -6.1± 0.9 | -52+7* |

Values are means ± S.E.M. for the number of rats given in parentheses. The control values for MAP, HR and striatal DA release are 97 ± 10 mm Hg, 410 ± 16 beats/min, and 209 ± 19 nmol, respectively. Δ = Difference between control values before and 10 min after the start of dl-THP injection. *$p < 0.05$, significantly different from control values (vehicle), ANOVA.

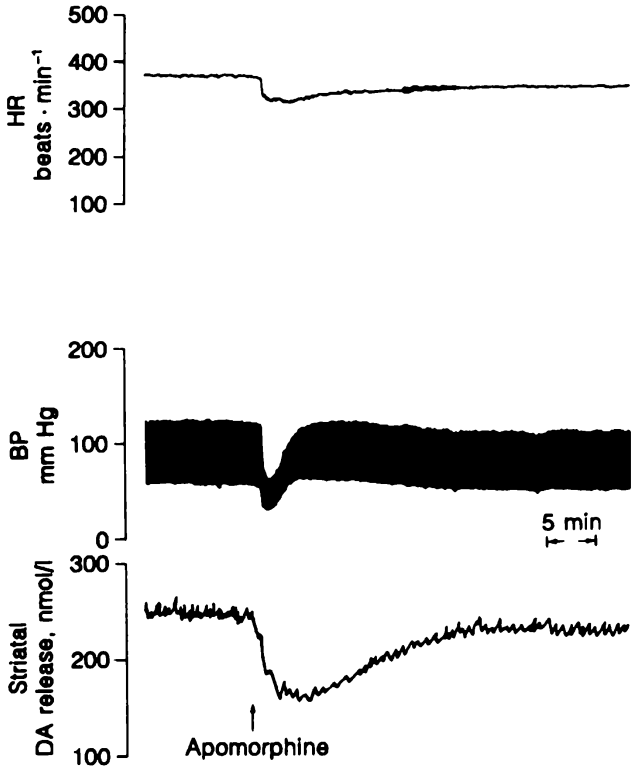


*Figure 5.* The time course changes in arterial blood pressure (BP), heart rate (HR), and striatal DA release produced by intravenous administration of apomorphine (0.5 mg/kg) in an anesthetized rat.

**TABLE IV**

Maximal changes in both MAP and HR produced by intravenous injection of dl-THP in rats with different preparations. Values are means ± S.E.M. of 8 rats. The control values for MAP and HR, respectively, are 90 ± 5 mm Hg, 357 ± 13 beats/min for sham-operated rats, 110 ± 7 mm Hg and 416 ± 15 beats/min for vagotomized rats, and 60 ± 4 mm Hg and 319 ± 12 beats/min for spinal rats. $p < 0.05$, significantly different from corresponding control values (sham-operated rats), ANOVA.

| Treatment (mg/kg) | △MAP mmHg | △HR beats/min |
|---|---|---|
| Sham-operated rats | | |
| 1 | -15.4± 2.1 | -14.7± 1.7 |
| 5 | -25.2± 2.7 | -30.5± 4.9 |
| Spinal rats | | |
| 1 | -3.3± 2.1* | -13.8± 1.6 |
| 5 | -5.6± 2.2* | -29.7± 3.8 |
| Vagotomized rats | | |
| 1 | -14.3± 2.3 | -4.2± 1.5* |
| 5 | -23.5± 2.4 | -6.6± 2.3* |

**TABLE V**

Effects of intravenous injection of saline, amphetamine, apomorphine, haloperidol or dl-THP on MAP HR and striatal DA release in rats. Values are means ± S.E.M. of 8 rats. *$p < 0.05$, significantly different from corresponding control values (sham operated rats), ANOVA.

| Treatment (mg/kg) | △MAP (mmHg) | △HR (beats/min) | △Striatal DA release (nmol/l) |
|---|---|---|---|
| 0.9 % Saline | 4± 2 (n=7) | -3± 1 (n=7) | -4± 2 (n=2) |
| Amphetamine | | | |
| 0.5 | 32± 4 (n=8) | 42± 5 (n=8) | 55± 6 (n=8)* |
| 1.25 | 48± 6 (n=8)* | 71± 7 (n=8)* | 186± 22 (n=8)* |
| Apomorphine | | | |
| 0.5 | -33± 5 (n=6)* | -66± 8 (n=6)* | -72± 9 (n=6)* |
| 1.25 | -54± 6 (n=6)* | -105± 14 (n=6)* | -128± 13 (n=6)* |
| Haloperidol | | | |
| 0.5 | -13± 2 (n=7)* | -15± 2 (n=7)* | 85± 9 (n=7)* |
| 1.25 | -47± 5 (n=8)* | -62± 6 (n=8)* | 288± 32 (n=8)* |
| 0.9% saline + dl-THP 10 mg/kg | -31± 3 (n=6) | -43± 6 (n=6)* | 162± 15 (n=6) |
| Amphetamine 0.5 mg/kg + dl-THP 10 mg/kg | 3± 2 (n=6)** | 4± 2 (n=6)** | 221± 27 (n=6)** |

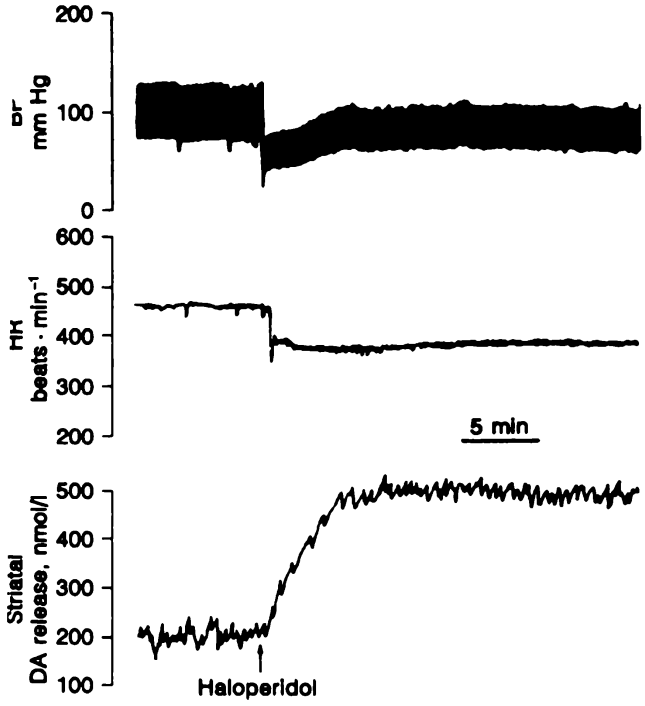


*Figure 6.* The time course changes in arterial blood pressure (BP), heart rate (HR), and striatal DA release produced by intravenous administration of haloperidol (1.25 mg/kg) in an anesthetized rat.

**TABLE VI**

Maximal changes in mean arterial pressure (MAP), heart rate (HR) and hypothalamic 5-HT release produced by intravenous injection of dl-THP in anesthetized rats. Values are means ± S.E.M. of 8 rats. Δ =Difference between control values before and 10 min after the start of dl-THP injection. *p < 0.05, significantly different from control values (vehicle), ANOVA.

| Treatment (mg/kg) | △MAP (mmHg) | △HR (beats/min) | △Hypothalamic 5-HT release(% baseline) |
|---|---|---|---|
| Vehicle | 4± 1 | -3± 1 | 4± 1 |
| 1 | -11± 3* | -11± -2* | -21± 4* |
| 5 | -21± 3* | -38± 5* | -39± 5* |
| 10 | -38± 4* | -43± 7* | -52± 7* |

## TABLE VII

Maximal changes in MAP and HR produced by intravenous injection of DOI, cyproheptadine or dl-THP in anesthetized rats. Values are means ± S.E.M. of 8 rats. The control values for MAP and HR are, respectively, 90 ± 5 mm Hg, 357 ± 13 beats/min for sham operated rats, 110 ± 7 mm Hg and 416 ± 15 beats/min for vagotomized rats, and 60 ± 4 mm Hg and 319 ± 12 beats/min for spinal rats. $p < 0.05$, significantly different from corresponding control values (sham operated rats), ANOVA. # p , 0.05 significantly different from corresponding DOI, 250 μ g/kg., i.v. values, ANOVA.

| Treatment (mg/kg) | Parameter measured | |
|---|---|---|
| | △MAP (mmHg) | △HR (beats/min) |
| 0.9% Saline, i.v. | 7± 3 | 4± 2 |
| DOI, 10 μg/kg, i.v. | 14± 1* | 15± 2* |
| DOI, 50 μg/kg, i.v. | 30± 5* | 30± 8* |
| DOI, 250 μg/kg, i.v. | 51± 9* | 52± 8* |
| Cyproheptadine, 2 mg/kg, i.v. | -24± 3* | -66± 13* |
| Cyproheptadine, 5 mg/kg, i.v. | -46± 3* | -158± 24* |
| Ketanserin, 2 mg/kg,, i.v. | -25± 4* | -63± 7* |
| Ketanserin, 5 mg/kg,, i.v. | -51± 5* | -164± 35* |
| 0.9% saline + dl-THP, 5 mg/kg | -25± 4 | -32± 6 |
| DOI, 250 μg/kg + dl-THP, 5 mg/kg | 6± 3# | 38± 11# |

**TABLE VIII**

The effect of dl-THP on colonic temperatures and hypothalamic serotonin (5-HT) release in hyperthermia induced by lipopolysaccharide (LPS) and interleukin -1β (IL-1β). Dl-THP and aspirin were injected 30 min after LPS (μg/kg) intraperitoneally (i.p.) injected or 180 min after IL-1β (10 ng/kg) intracerebroventricularly (i.c.v.) injected. The values are means ± S.E.M. for 8 rats per group. Δ represents the difference between the control values before injection and the maximum change after injection. Significant differences from the corresponding control values (saline group) are represented by * $p < 0.05$, ** $p < 0.01$ by ANOVA.

| Treatment | Change in colonic temperature (Δ°C) | | |
|---|---|---|---|
| | Normal | LPS | IL-1β |
| Saline 0.9% (i.p.) | 0.15±0.05 | 1.51±0.10 | 2.36±0.32 |
| dl-THP | | | |
| 10.0 mg/kg (i.p.) | -1.06±0.22* | 0.60±0.24* | 1.27±0.33** |
| 20.0 mg/kg (i.p.) | -1.78±0.23** | -0.39±0.19** | 0.75±0.21** |
| Aspirin | | | |
| 75 mg/kg (i.p.) | -0.12±0.13 | 0.42±0.17* | 1.02±0.12* |
| 150 mg/kg (i.p.) | -0.21±0.21 | 0.14±0.09** | 0.72±0.21** |

induced depressor response. On the other hand, bilateral vagotomy, but not spinal transection, completely abolished dl-THP-induced bradycardia.

The effects of intravenous administration of amphetamine, apomorphine or haloperidol on cardiovascular parameters and striatal DA release in rats are summarized in Table V. Administration of amphetamine (0.5-1.25 mg/kg) produced hypertension, tachycardia and increased striatal DA release, while administration of apomorphine (0.5-1.25 mg/kg) produced hypotension, bradycardia and decreased striatal DA release (Figure 5). However, administration of haloperidol produced hypotension, bradycardia and increased striatal DA release (Figure 6). In addition, amphetamine (0.5 mg/kg) completely reversed the dl-THP (10 mg/kg)-induced hypotension and bradycardia in rats (Figure 4), although DA release was further increased during the combined treatment.

Table VI summarizes the response of cardiovascular parameters and hypothalamic 5-HT release produced by intravenous administration of vehicle or dl-THP in rats under urethane anesthesia. Administration of dl-THP (1-10 mg/kg), but not vehicle, produced a dose-related fall in either mean arterial pressure, heart rate or hypothalamic 5-HT release. A typical example for this kind of response is shown in Figure 7. For a dose of 10 mg/kg of dl-THP, either the mean arterial pressure, heart rate or hypothalamic 5-HT release fell immediately after dl-THP injection and did not return to preinjection level even after 60 min. dl-THP treatment produced an insignificant change in either $PaCO_2$, $PaO_2$ or pH (data are not shown).

Table VII summarizes the response of cardiovascular parameters produced by the intravenous administration of DOI, cyproheptadine or ketanserin to rats. Intravenous administration of cyproheptadine (2 and 5 mg/kg) or ketanserin (2 and 5 mg/kg) caused a decrease in both mean arterial pressure and heart rate. In contrast, intravenous injection of DOI (10-250 mg/kg) caused an increase in both mean arterial pressure and heart rate. Furthermore, the dl-THP-induced hypotension and bradycardia were reversed by DOI treatment.

*Discussion*

As indicated from the results in Figure 1, dl-THP significantly decreased motor activity and showed a dose-response pattern. However, rats treated with dl-THP at 20 mg/kg produced a rigid syndrome. This result shows that the observation that dl-THP has a sedative effect at those dosages less than 10 mg/kg and a rigid effect at those dosages greater than 20 mg/kg. Therefore, the sedative mechanism and rigid effect of dl-THP were investigated by measuring the motor activity and concentration of central monoamines and its metabolites In order to investigate the relationship of the sedative effect of dl-THP and monoaminergic activity, we used drugs (α-MT, haloperidol and l-DOPA plus benserazide), that have effects on the central monoaminergic system, which can produce behavior changes in rats. Generally, α-MT (an inhibitor of tyrosine hydroxylase) pretreatment inhibits the newly-synthesized pool of catecholamines (Widerlov and Lewander, 1978a); haloperidol, the dopaminergic receptor blockers (Megens *et al.*, 1988). Therefore, these two compounds reduce motor activity. Whereas

apomorphine (a DA receptor agonist) (Montanaro *et al.*, 1983) produces hypermotility. dl-THP significantly augmented the hypomotility produced by α-MT or haloperidol, and reduce the hypermotility produced by l-DOPA plus benserazide. These facts imply that the sedative effects of dl-THP may be involved in the decrease in central dopaminergic activity.

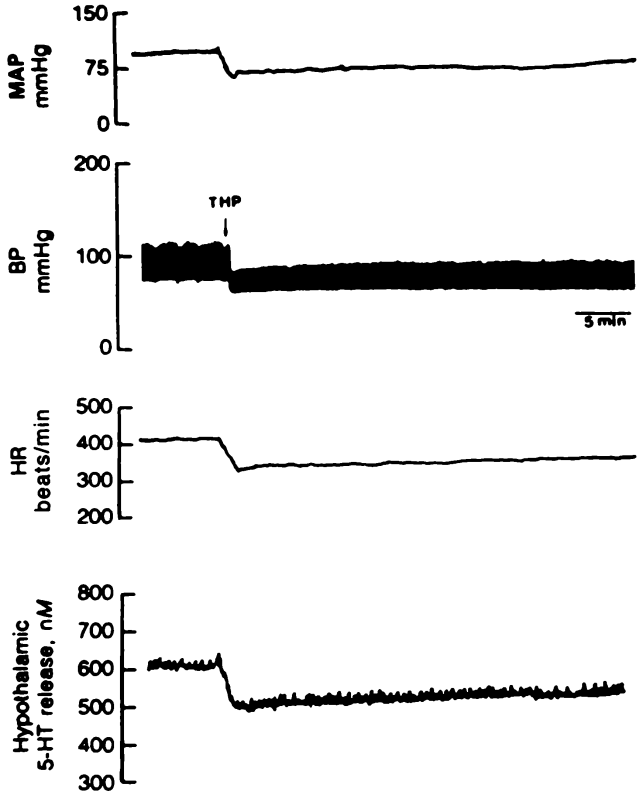


*Figure 7.* The time course changes in arterial blood pressure (BP), heart rate (HR), and hypothalamic serotonin (5-HT) release produced by intravenous administration of dl-THP (10 mg/kg) in an anesthetized rat.

In order to understand the relationship between the sedative effect of dl-THP and the levels of monoamines and its metabolites, we estimated the levels of the central monoamines and its metabolites in rats. We found that dl-THP (10 mg/kg, i.p.) significantly decreased the levels of NE ($p < 0.01$) and DA ($p < 0.05$) and increased the level of HVA ($p < 0.05$) in the cortex and brain stem. dl-THP increased the ratio of VMA/NE, HVA/DA in the cortex and brain stem. These results indicated that dl-THP increased the NE and DA turnover rate in the cortex and brain stem. Generally, because DA is an excitatory neurotransmitter in the cortex and brain stem (Beninger, 1983), the NE and DA levels of cortex and brain stem were decreased. The present results demonstrate that the inhibitory effect of dl-THP on motor activity in rats was related to the decrease of DA levels in the cortex and brain stem.

We observed the behavioral changes of dl-THP by naive-behavioral observations, and found that dl-THP produced rigid syndrome at 20 mg/kg dosage. In order to understand the relationship of the rigid effect of dl-THP and the levels of monoamines and its metabolites, we also detected the levels of the central monoamines and its metabolites in rats. We found that dl-THP (20 mg/kg, i.p.) significantly decreased the level of NE, DA ($p < 0.01$) in the cortex and brain stem and 5-HT ($p < 0.01$) in the cortex, and increased the level of HVA ($p < 0.01$) in the cortex and brain stem and 5-HIAA ($p < 0.01$) in the cortex. dl-THP increased the ratio of VMA/NE, HVA/DA in the cortex and brain stem and 5-HIAA/5-HT in the cortex. From these results, it was indicated that dl-THP increased the NE and DA turnover rates in the cortex and brain stem, and 5-HT turnover rate in the cortex. Generally, the more decreases in the activities of central monoaminergic system, the more rigid syndrome was produced (Prezwicoka *et al.*, 1977; Pycock, 1977). These results indicated that the rigid effect of dl-THP at 20 mg/kg dosage may be related to the decrease in central dopaminergic activity in the cortex and brain stem and central serotonergic activity in the cortex.

The present results show that the intravenous administration of dl-THP or haloperidol produced hypotension, bradycardia and increased striatal DA release in rats. The dl-THP-induced hypotension was attenuated by spinal transection or amphetamine treatment, while the dl-THP-induced bradycardia was attenuated by bilateral vagotomy or amphetamine treatment. Administration of amphetamine alone produced hypertension, tachycardia and increased striatal DA release. Thus, it appears that dl-THP shares with haloperidol (a dopaminergic $D_2$ antagonist that can block both post- and pre-synaptic receptors) the same depressor mechanisms in rats.

In fact, biochemical and behavioral experiments have suggested that dl-THP presents all the characteristics of a DA $D_2$ receptor antagonist (Jin *et al.*, 1983; Jin *et al.*, 1985; Xu *et al.*, 1985) supporting the present results. Binding experiments have shown that dl-THP can competitively inhibit $^3$H-spiperone binding to DA $D_2$ receptors in homogenates of calf striatum (Xu *et al.*, 1989). Other studies have demonstrated that dl-THP has a stimulating effect on rat tyrosine hydroxylase activity via a feedback control from presynaptic DA receptors and that it can reverse the inhibitory effect of apomorphine on tyrosine hydroxylase activity (Jin *et al.*, 1985). Behavioral experiments have also revealed that dl-THP can antagonize the stereotyped behavior induced by apomorphine and amphetamine in 6-hydroxydopamine (6-OHDA) unilaterally lesioned rats. Furthermore, dl-THP induced an increase in extracellular DA concentration, reversed the decrease in extracellular DA concentration induced by apomorphine, and enhanced the effects of the electrical stimulation of the nigrostriatal pathway.

Recently, our results showed that stimulation of the SNC, in addition to enhancing the DA release in the corpus striatum, elicited proportional hypertension and tachycardia in rats (Lin and Yang, 1994). The SNC stimulation-induced hypertension and tachycardia were attenuated by prior destruction of the nigrostriatal DA system produced by intramedial forebrain bundle injection of 6-OHDA and by prior blockade of postsynaptic

DA receptors produced by intra-striatal injection of DA release antagonists, haloperidol and pimozide. The SNC stimulation-induced hypertension was attenuated by spinal transection, whereas the SNC stimulation-induced tachycardia was attenuated by bilateral vagotomy. Local injection of either DA or apomorphine into the rat striatum was also shown to elicit both hypertension and tachycardia, probably by acting on postsynaptic DA receptors in the corpus striatum. Therefore, in the present study, administration of either dl-THP or haloperidol may have blocked both the pre- and post-synaptic dopaminergic $D_2$ receptors in the corpus striatum of the rat brain and resulted in both hypotension, bradycardia and increased striatal DA release. Amphetamine administration, in the present study, may have increased DA release in the corpus striatum and thus resulted in hypertension and tachycardia. Accordingly, amphetamine treatment reversed the dl-THP-induced hypotension and bradycardia in rats. However, intravenous administration of apomorphine may have stimulated the presynaptic DA autoreceptors and resulted in decreased striatal DA release (Jin *et al.* 1985), hypotension and bradycardia in rats.

The present results demonstrate that intravenous injection of dl-THP elicited a proportional decrease in either the mean arterial pressure or heart rate, which could be mimicked by intravenous injection of 5-$HT_2$receptor antagonists such as cyproheptadine and ketanserin in rats. The 5-$HT_2$-receptor agonist DOI, in addition to producing hypertension and tachycardia, attenuated the dl-THP-induced hypotension and bradycardia in rats, when given intravenously. In addition, dl-THP administration caused a proportional decrease in the endogenous release of 5-HT in the hypothalamus. These results suggest that dl-THP decreases the hypothalamic 5-HT release and results in a decrease in either the mean arterial pressure or the heart rate in rats. Our recent results also showed that the hypotension induced by dl-THP was attenuated by pretreatment of animals with spinal transection, whereas the bradycardia induced by dl-THP was attenuated by bilateral vagotomy (Chueh *et al.*, 1995). These observations imply that the dl-THP-induced hypotension is attributable to inhibition of sympathetic efferent activity, while the dl-THP-induced bradycardia is attributable to activation of parasympathetic efferent activity.

The proposed contention is supported by many previous findings. For example, intracerebral injection of 5-HT or 5-$HT_2$-receptor agonists resulted in an increase in sympathetic outflow to the heart and rise in arterial blood pressure (Minson *et al.*, 1990; Gillis *et al.*, 1992; Kristic and Djurlpvic, 1980), while intracerebral injection of ketanserin decreased arterial blood pressure (Mandal *et al.*, 1990). The pressor effects of raphe nuclei stimulation were also shown to be abolished by transection to the midbrain, confirming the ascending projection pathway (Kuhn *et al.*, 1980). Transection caudal to the midbrain also abolished these pressor responses and demonstrated that a relay descending pathway must be activated for sympathetic activity to be modified. The present results showed that dl-THP decreased the serotonin release in the hypothalamus and shared with the 5-$HT_2$ antagonists the same depressor action. These results suggest

that the dl-THP-induced hypotension and bradycardia may be due to alteration in the 5-HT releasing process in the hypothalamus. However, in additional to central action, dl-THP may possess peripheral action. It was found that in pitched rats, I-THP administered into the common carotid artery reduced the 5-HT *(i.v.)*-induced increase in diastolic pressure (Zhao *et al.* 1988). The contraction of the isolated blood vessels induced by 5-HT, norepinephrine or KCI was also antagonized by l-THP. In order to ascertain the possible site of action of dl-THP on the 5-HT synapse in the central nervous system, future experiments should be carried out to assess the effects of intracerebral injection of a water soluble form of dl-THP on cardiovascular responses. The dl-THP used in the present study was dissolved in phosphoric acid and therefore not suitable for central injection.

In conclusion, the sedative mechanism of dl-THP at low dosage may be related to the decrease in the dopaminergic activity in the cortex and the brain stem. The rigid side effect of dl-THP may be related to the decrease of the dopaminergic activity in the cortex and brain stem and serotonergic activity in the cortex. dl-THP induces hypotension and bradycardia through a serotonergic release process in the hypothalamus and through DA D2 receptor antagonism in the striatum in rats.

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*References*

Beninger, R.J. (1983). The role of dopamine in locomotor activity and learning. Brain Res. Rev. 6:173-196.

Brazell, M.P., Kasser, R.J., Rennet, K. J, Feng, J., Moghaddam, B. and Adams, R.N. (1987). Electrocoating carbon fiber electrodes with nation improve selectivity for electroactive neurotransmitters. J. Neurosci. Methods 22:167-172.

Chueh, F.Y., Hsieh, M.T., Chen, C.F. and Lin, M.T. (1995). dl-Tetrahydropalmatine-produced hypotension and bradycardia in rats through the inhibition of central nervous dopaminergic mechanisms. Pharmacology 51: 237-244.

Cornish, J.L., Van den Buuse, M. (1994). Presser responses to electrical and chemical stimulation of the rat brain A 10 dopaminergic system. Neurosci. Lett. 176:142-146.

Dharmananda, S. (1994). The story of JIN BU HUAN. Herbal Gram 31: 28-31.

Gillis, R.A., Mandal, A.K., Hernandez, Y.M., Norman, W.P., Dretchen, K.E. and Travagli, R.A. (1992). Brainstem serotonin receptor subtypes and cardiovascular control. Adv. Biosci. 85: 361-381.

Gonon, F., Fombarlet, C.M., Buda, M. and Pujol, J.F. (1981) Electrical treatment of phrolytic carbon fiber electrodes. Anal. Chem. 53:1386-1390.

Gothert, M. and Kolassa, N. (1990). 5-HT,A receptors in the brain: a subtype of serotonin receptors involved in blood pressure reduction. J. Cardiovasc. Pharmacol. 7: sl-s93.

Hsu, B. and Kin, K.C. (1962). Pharmacological study oftetrahydropalmatine and its analogs. Arch. Int. Pharmacodyn. Ther. 139:318-327.

Hsu, B. and Kin, K.C. (1964). Further studies on the pharmacology of tetrahydropalmatine and its analogs. Int. J. Neuropharmacol. 2:283-290.

Jin, G.Z., Xu, J., Zhang, F.T., Yu, L.P., Li, J.H. and Wang, X.L. (1983). Relevance of the sedativetranquilizing effect of ltetrahydropalmatine to brain monoaminergic neurotransmitters. Acta Pharmacologica Sinica 4: 4-10.

Jin, G.Z., Kin, K.C., Wang, X.L. and Shi, W.X. (1985). Tetrahydroprotoberberine. A new chemical type of antagonist of dopamine receptor. Sci. Sin. 10: 925-930.

Jin, G.Z., Xu, S.X. and Yu, L.P. (1985). Different effects of l- and d-tetrahydropalmatine on dopaminergic system in rat's brain. Sci. Sin. 11:1015-1022.

Kin, K.C. and Hsu, B. (1957). Study on the pharmacological actions of *Corydalis* I. Analgesic action of *Corydalis* A, B and L. Acta. Physiol. Sin. 21:150-157.

Kirk, R.E. (1986). *Experimental Design: Procedures for the Behavioral Sciences.* Book/Cole, Belmont, pp 72-75.

Kristic, M. and Djurlpvic, D. (1980). Analysis of cardiovascular response to central administration of 5-hydroxytryptamine in rats. Neuropharmacology 19: 455-463.

Kuhn, D.M., William, A., Wolf, B.A. and Lovenberg, W. (1980). Review of the role of the central serotonergic neuronal system in blood pressure regulation. Hypertension 2: 243-255.

Kuhn, D.M., Wolf, W.A. and Lovenberg, W. (1980). Pressor effects of electrical stimulation of the dorsal and median raphe nuclei in anesthetized rats. J. Pharmacol. Exp. Ther. 214: 403-409.

Lin, M.T. and Yang, J.J. (1994). Stimulation of the nigrostriatal dopamine system produces hypertension and tachycardia in rats. Am. J. Physiol. 226:H2489-H2496.

Liu, D. and Zhao, G.S. (1987). Effects of dl-tetrahydropalmatine on cardiac hemodynalnics in dogs. Yao Hsueh Hsueh Pao 22:537-540.

Liu, G.Q. (1983). Influence of pargyline on depletion of catecholamines in the rat brain caused by dl-tetrahydropalmatine. Yao Hsueh Hsueh Pao, 8: 472-474.

Liu, G.Q., Algeris, A. and Garattini, S. (1982). dl-Tetrahydropalmatine as monoamine depletor. Arch. Int. Pharmacodyn. Ther. 258: 39-50.

Mandal, A.K., Kellar, K.J., Norman, W.P. and Gillis, R.A. (1990). Stimulation of serotonin 2 receptors in the ventrolateral medulla of the cat results in nonuniform increases in sympathetic outflow. Circ. Res. 67: 1267- 1280.

Marcenac, F., Jin, G.Z. and Gonon, F. (1986). Effect of l-tetrahydropalmatine on dopamine release and metabolism in the rat striatum. Psychopharmacology 89:89-93.

Megens, A.A., Awouters, F. and Niemegeers, H. (1988). Differential effects of the new antipsychotic risperidone on large and small motor movements in rats: a comparison with haloperidol. Psychopharmacology 95: 493-496.

Minson, J., Chalmers, J., Drolet, G., Kapoor, V., Llewellyn-Smith, I., Mills, E., Morris, M. and Pilowsky, P. (1990). Central serotonergic mechanisms in cardiovascular regulation. Cardiovasc. Drugs Ther. 4: 27-32.

Motanaro, N., Vaccheri, A., Dall'Olio, R. and Gandolfi, O. (1983). Time course of rat motility response to apomorphine: A simple model for studying preferential blockade of brain dopamine receptors mediating sedation. Psychopharmacology 81: 214-219.

Paxinos, G. and Watson, C. (1982). *The Rat Brain in Stereotaxic Coordinates.* Academic Press, New York, pp 36-46.

Przewicoka, B., Kukuika, L. and Tatarczyinska, E. (1977). The effect of lesions of raphe nuclei on the cataleptic action of neuroleptics. Pol. J. Pharmacol. & Pharm. 29: 581-589.

Pycock, C. (1977). Noradrenergic involvement in dopamine-dependent stereotyped and cataleptic responses in the rat. Naunyn-Schmiedebergs Arch. Pharmacol. 298: 15-22.

Shibuya, T., Sato, K. and Salafsky, B. (1982). Simultaneous measurement of biogenic amines and related compounds by high performance liquid chromatography. Int. J. Clin. Pharmacol. Ther. Toxicol. 20: 297-302.

Wang, Y. and Li, D.X. (1987). Antiarrhythmic action of l-tetrahydropalmatine. Acta Pharmacol, Sin. 8:337-340.

Widerlov, E. and Lewander, T. (1978a). Inhibition of the *in vivo* biosynthesis and changes of catecholamine levels in rats brain after -methyl-p-tyrosine; time- and dose- response relationships. Naunyn-Schmiedebergs Arch. Pharmacol. 304: 111-123.

Xu, S.X., Chen, Y., Jin, G.Z. (1985). Comparison of affinities of tetrahydroprotoberberine on dopamine receptor in calf caudatum. Kexue Tong Pao 30:468-471.

Xu, S.X., Xu, L.P., Han, Y.R., Chen, Y. and Jin GZ (1989). Effects of tetrahydroprotoberberine on dopaminergic receptor subtypes in brain. Acta Pharmacol. Sin. 10:104-110.

Zhao, G.J., Ren, S.L., Wu, S. and Yu, L.S. (1988). Effect of l-THP on density of blood vessels induced by 5-hydroxytryptamine. Chin. J. Pharmacol. Toxicol. 2: 247-251.

Zhu, D.Y. (1987). Recent advances on the active components in Chinese medicine. ACME 1: 251-286.